RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 2-50 are pending. Claim 1 was canceled and claims 36-45 were withdrawn as drawn to a nonelected invention. Claims 2-35 and 46-50 were examined in the April 21, 2008 Office Action. With this Amendment and Response, claims 16 and 17 are amended to more particularly point out and distinctly claim the invention.

B. Rejections under 35 U.S.C. 103(a)

(1) Claims 2-35 and 46-50 were rejected under 35 U.S.C. 103(a) as being unpatentable over Yu *et al.*, 2000, *Plant Physiology* 124:781-793 (Yu) in view of Wisman *et al.*, 1998, *Proc. Natl. Acad. Sci. U.S.A.* 95:12432-12437 (Wisman).

As an initial matter Applicants note that the Office Action of April 21, 2008 was made final even though the instant rejection constitutes a new rejection not based on claim amendments made in the Amendment and Response of January 30, 2008. Applicants therefore request a withdrawal of the finality of the current Office Action and a refund of the RCE fee.

The Action states that Yu teaches production of the isoflavonoid genistein in a non-legume dicot and monocot plant and plant transformed with isoflavone synthase and upregulating the phenylpropanoid pathway to increase genistein production in the presence of isoflavonoid synthase relative to a non-upregulated control. The Action acknowledges that Yu does not teach regulating flavanone 3-hydroxylase. The Action further states that Wisman teaches a *tt6* mutant in *Arabidopsis* where the *tt6* gene comprises a mutant flavanone 3-hydroxylase that has lost its function of converting its substrate into product thereby allowing for the accumulation of naringenin. The Action concludes that it would have been obvious to increase isoflavone synthase expression in a plant to increase isoflavonoid biosynthesis, in light of the teachings of Yu. The Action further concludes that addition of the mutant flavanone 3-hydroxylase to eliminate the

flavanone 3-hydroxylase branch of the pathway would be expected to lead to the accumulation of isoflavanoids due to the accumulation of their precursor naringenin.

Applicants traverse this rejection. While Yu shows that increasing the presumed levels of upstream substrates in the phenylpropanoid pathway by environmental stress leads to small increases in isoflavone production in plants engineered to express an isoflavone synthase transgene. In the instant application, it was not known how down-regulating one branch of the phenylpropanoid pathway affects flux through other branches. Further, regulation of the phenylpropanoid pathway in plants is sufficiently complex such that there would not have been a reasonable expectation that down-regulating flavanone 3-hydroxylase in a plant would increase isoflavonoid biosynthesis. Neither Yu nor Wisman provide further elucidation into the relevant controls.

To illustrate the unpredictability of the regulation of plant natural product pathways including phenylpropanoid pathway regulation, three examples are provided to illustrate the point that the regulatory architecture of those pathways cannot be predicted simply by expecting that a buildup of a particular intermediate would lead to an increase in metabolites of that intermediate that are along an unblocked pathway.

1. Effects of Overexpression of Phenylalanine Ammonia Lyase (PAL). PAL is the first enzyme in the phenylpropanoid pathway. Flavonoids, chlorogenic acid and lignin are all products of this pathway. Overexpression of PAL in transgenic tobacco results in a directly proportional increase in the level of chlorogenic acid, a less than proportional increase in flavonoids, and no increase in lignin (Howles *et al.*, 1996, *Plant Physiology* 112: 1617-1624, copy provided herewith). What would be predicted, however, is similar increases in all three when flux into the pathway is increased. Thus, overexpression of PAL exhibits control mechanisms that lead to unexpected downstream regulation.

2. Effects of Downregulation of Caffeic Acid-3-O-methyltransferase (COMT) or Caffeoyl CoA-3-O-methyltransferase (CCoAOMT). CCoAOMT and COMT catalyze, respectively, the first and second methylation steps in the biosynthesis of lignin monomers, through the phenylpropanoid pathway. Downregulating COMT would be predicted to reduce the amount of di-methylated lignin units (which it does), but not affect the levels of mono-methylated units (or perhaps increase them). In fact, both are decreased in transgenic alfalfa expressing a COMT antisense transgene. Likewise, down-regulating CCoAOMT would be predicted to result in reductions of both mono and di-methylated lignin monomers, when in fact, in alfalfa, it only reduces the levels of the mono-methylated units (Chen *et al.*, 2006, *Plant Journal* 48: 113-124). Thus, downregulation of COMT and CCoAOMT also exhibits control mechanisms that lead to unexpected downstream regulation.

3. Engineering Alkaloid Biosynthesis. Inventor Dixon recently noted that noted that

> recent examples of metabolic engineering within alkaloid pathways ..... suggest that much remains to be learned concerning pathway integration and cross-talk. For example, genetic down-regulation of berberine bridge enzyme (BBE) or N-methylcoclaurine 3'-hydroxylase in California poppy cell cultures by antisense expression resulted in decreased growth and, surprisingly, elevated levels of several amino acids but not tyrosine, the initial precursor of the benzophenathridines. No intermediates in the alkaloid pathway were observed to accumulate in the antisense lines. Over-expression of BBE in roots led to a large increase in the levels of dihydrochelilutine, only a minor benzophenathridine in control roots.

Dixon, 2005, *Current Opinion in Plant Biology* 8: 329-336. As an additional complicating factor, metabolic channeling may be a common feature of plant natural product pathways, and this phenomenon, which is hard to demonstrate experimentally other than by doing flux measurements, makes it virtually impossible to predict *a priori* how a pathway will respond to changes in the levels of upstream intermediates, or how effectively a transgene product will compete for substrate from the endogenous pathway (Winkel, 2004, *Annual Review of Plant Biology* 55: 85-107).

Because of the unpredictable nature of plant metabolic pathways, the large increase in isoflavonoids resulting from down-regulation of flavanone 3-hydroxylase in a transgenic plant expressing isoflavone synthase would not have been obvious and was unexpected because the controls on the phenylpropanoid pathway are unpredictable.

In light of the above discussion, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 2-35 and 46-50 under 35 U.S.C. 103(a) as being unpatentable over Yu in view of Wisman.

(2) Claims 2-35 and 46-50 were also rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 7,189,895 to McGonigle and Odell ("the '895 patent"), in view of WO 00/44909, and in further view of Applicants' asserted disclosure of the state of the prior art.

Although Applicants submitted a Declaration under 37 C.F.R. 1.131, with the Amendment and Response dated January 30, 2008, that established the currently claimed invention was made prior to the June 13, 2002 effective date of the '895 patent, the Action maintains the rejection, asserting that the instant claims interfere with the claims of the '895 patent, and urging that Applicants suggest an interference under 37 C.F.R. 41.202. Applicants assert that the instant claims do not interfere with the claims of the '895 patent.

37 CFR 41.203 defines interfering subject matter:

> "(a) Interfering subject matter. An interference exists if the subject matter of a claim of one party would, if prior art, have anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa."

Thus, the claims of the '895 patent interfere if those claims are obvious in light of the claims of the instant claims, ***and vice versa***.

The independent claims of the '895 patent are 1 and 12:

1. A method of increasing isoflavonoid production in an isoflavonoid-producing plant, the method comprising:

a) transforming a plant with

(1) a first recombinant DNA construct comprising a polynucleotide selected from the group consisting of:

(i) a polynucleotide encoding all or part of a flavanone 3-hydroxylase from the plant;

(ii) a polynucleotide comprising a 5′ non-coding sequence, a 3′ non-coding sequence, or both, of an isolated nucleic acid fragment which encodes a flavanone 3-hydroxylase from the plant; or

(iii) a polynucleotide comprising (i) and (ii); and

(2) at least one second recombinant DNA construct comprising a polynucleotide encoding a maize C1 myb transcription factor and a maize R myc-type transcription factor; and

b) growing the transformed plant of (a); and

c) evaluating the plant or plant part obtained from the transformed plant for an increased quantity of isoflavonoid in the transformed plant or plant part as compared to the plant or plant parts obtained from an untransformed plant.

12. An isoflavonoid-producing plant comprising in its genome

(1) a first recombinant DNA construct comprising a polynucleotide selected from the group consisting of:

i) a polynucleotide encoding all or part of a flavanone 3-hydroxylase from the plant;

(ii) a polynucleotide comprising 5′ non-coding sequence, 3′ non-coding sequence, or both, of an isolated nucleic acid fragment which encodes a flavanone 3-hydroxylase from the plant; or

(iii) a polynucleotide comprising (i) and (ii); and

(2) at least one second recombinant DNA construct comprising a polynucleotide encoding a maize C1 myb transcription factor and a maize R myc-type transcription factor;

wherein the plant or plant parts obtained from the transformed plant have an increased quantity of isoflavonoid as compared to the plant or plant parts obtained from an untransformed plant.

Thus, the claims of the '895 patent are directed to a method of increasing isoflavonoid production in a plant, and the plant itself, where the plant comprises (1) a polynucleotide encoding all or part of a flavanone 3-hydroxylase from the plant and control elements of that gene such that it is expressed; and (2) at least one second recombinant DNA construct comprising a polynucleotide encoding a maize C1 *myb* transcription factor and a maize R *myc*-type transcription factor.

The above claims do not interfere with the instant claims at least because the claims of the '895 patent recites the expression of maize C1 *myb* transcription factor and a maize R *myc*-type transcription factor whereas the instant claims recite up-regulating isoflavone synthase by introducing a transgene encoding the isoflavone synthase into the plant.

The '895 patent describes the maize C1 *myb* transcription factor and maize R *myc*-type transcription factor, and their effect on phenylpropanoid pathways at, *inter alia*, col. 2, line 40 - col. 3, line 39. C1 is described therein as a "transcription factor that regulates expression of genes involved in anthocyanin production and accumulation." C1 requires an R-type factor to activate target gene promoters. C1 was also known to regulate expression of phenylpropanoid pathway genes, leading to production of anthocyanins or flavonols. However, it was not known which phenylpropanoid genes are activated by C1, and the '895 patent does not shed light on this. Given the complex and unpredictable regulation of phenylpropanoid pathways as further discussed under **B.(1)** above, it could not be assumed that C1 activation leads to changes in phenylpropanoid, including isoflavonoid, accumulation by increasing expression of isoflavone synthase. Thus, the skilled artisan would not be led by the '895 to understand that increasing isoflavone synthase was the effect that caused C1 activation to accumulate isoflavonoids. Therefore, the instant claims are not obvious in light of the '895 patent.

Similarly, the instant specification would not lead the skilled artisan to believe that activating C1 would cause increased isoflavonoid accumulation ***by increasing isoflavone synthase activity*** because it is not known which genes C1 activates. Therefore, the claims of the '895 patent are not obvious in light of the instant specification.

Based on the above discussion, the instant claims are not obvious in light of the '895 patent, and the claims of the '895 patent are not obvious in light of the instant application. To interfere under 37 CFR 41.203, ***both*** the instant claims must be obvious in light of the '895 patent ***and*** the claims of the '895 patent must be obvious in light of the instant specification. Since ***neither*** of these conditions occur, there is no interference.

Because there is no interference, Applicants again assert that the '895 patent is not prior art for this rejection because the claimed invention was invented prior to the priority date of the '895 patent. Withdrawal of the rejection is thus respectfully requested.

CONCLUSION

In light of the above amendments and discussion, applicants respectfully request withdrawal of all rejections and examination of withdrawn claims 37-45, since those claims are dependent on allowable claim 24 or 40.

Respectfully submitted,

/ Robert E. Hanson/

Robert E. Hanson
Reg. No. 42,628
Attorney for Applicants

Sonnenschein Nath & Rosenthal LLP
1717 Main Street, Suite 3400
Dallas, TX 75201
Tel.: 214.259.0931
Fax: 214.259.0910

Date: October 28, 2008